CHAPTER 6

NOVEL INSIGHTS ON IRON DEFICIENCY IN HEART FAILURE

IJsbrand T. Klip
Adriaan A. Voors
Carolyn S. Lam
Dirk J. van Veldhuisen
Peter van der Meer

Submitted

ABSTRACT

Iron deficiency has recently been recognized as an individual comorbidity in patients with heart failure (HF). In both acute and chronic HF the prevalence of iron deficiency is high. Apart from its major role in the development of anemia, iron deficiency contributes to impaired cardiac and peripheral muscle dysfunction, leading to increased morbidity and mortality in patients with HF. However, current markers of iron status are not optimal to define iron deficiency in the context of HF and novel markers may be needed to aid in the diagnosis. Finally, a number of randomized controlled trials have shown favourable effects of intravenous iron on multiple clinical endpoints including exercise capacity, left ventricular function, quality of life and renal function in patients with HF and iron deficiency, irrespective of hemoglobin levels. The present review focuses on recent findings regarding global prevalence and prognostic consequences of iron deficiency in HF, current insights in iron homeostasis, novel diagnostic tools to define iron deficiency in HF, and provide an overview of both recent, ongoing and emerging therapeutic approaches to treat iron deficiency in HF.

ABBREVIATIONS

%HYPO	=	Percentage of hypochromic red blood cells
6MWT	=	6-minute walking test
CHr	=	Reticulocyte hemoglobin content
CKD	=	Chronic kidney disease
DMT1	=	Divalent metal transporter 1
ESAs	=	Erythropoietin stimulating agents
ESC	=	European society of cardiology
FCM	=	Ferric carboxymaltose
Fe-S	=	Iron-sulphur
HF	=	Heart failure
IRPs	=	Iron regulatory proteins
MCV	=	Mean corpuscular volume
NYHA	=	New York Heart Association
RDW	=	Red cell distribution width
RES	=	Reticuloendothelial system
ROS	=	Reactive oxygen species
sTfR	=	Soluble transferrin receptor
TfR1/2	=	Transferrin receptor 1/2
TIBC	=	Total iron binding capacity
TSAT	=	Transferrin saturation

INTRODUCTION

In the past decades, our gain in understanding the pathophysiology of heart failure (HF) has led to remarkable advances in treatment possibilities. However, despite these improvements, HF prevalence is rising and only small prolongations in survival are observed; 5-year mortality is still approximately 50%.1 In addition, normal daily activities of many patients remain restricted and quality of life is substantially impaired.2

The presence of comorbidities in patients with HF, especially with a preserved ejection fraction, is one of the factors that contribute to the negative impact of HF on multiple levels.3 Anemia and iron deficiency are common comorbidities in HF that can complicate treatment and affect clinical outcome.4–6 Iron deficiency is the most common nutritional disorder in the world and one of the leading risk factors for disability and death worldwide, affecting an estimated 2 billion people.7,8 Aside from traditionally linked to anemia, iron deficiency is also a separate condition with high prevalence, serious clinical consequences and poor prognosis. Whereas anemia in the setting of HF has received a lot of attention in the last decade, iron deficiency has only been recently identified as an important and frequently observed comorbidity in HF patients with and without concomitant anemia or renal dysfunction (*Figure 1*).

Although just recently recognized as an individual comorbidity in HF, iron deficiency is still frequently overlooked and often only explored in the context of anemia.9 Furthermore, current markers of iron status are not

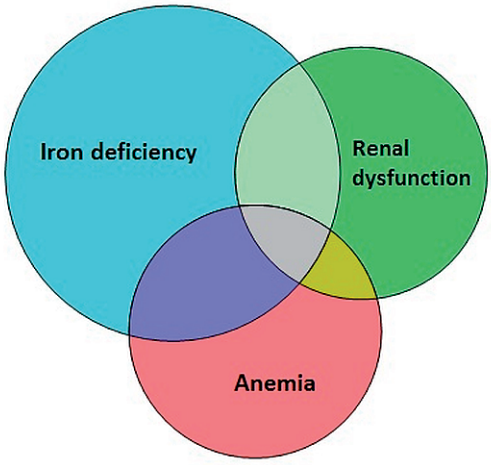

Figure 1. The interaction between iron deficiency, anemia and renal dysfunction in heart failure. *Adapted from Klip et al.*5

optimal to define iron deficiency in some chronic diseases (like HF) and require different cut-off values compared to healthy individuals. Finally, questions concerning the use and modality of iron supplementation to treat iron deficiency in HF remain a topic of controversy. The importance of iron deficiency and possible treatment options in HF therefore merit more clinical awareness. In this review, we will focus on recent advances made in the pathophysiology of iron homeostasis in HF, discuss contemporary and possible future diagnostic tools to define iron deficiency in HF, highlight global perspective of iron deficiency in HF with regard to clinical implications and prognostic consequences, and provide an overview of recent, ongoing and emerging future therapeutic approaches to treat iron deficiency in HF (*Figure 2*).

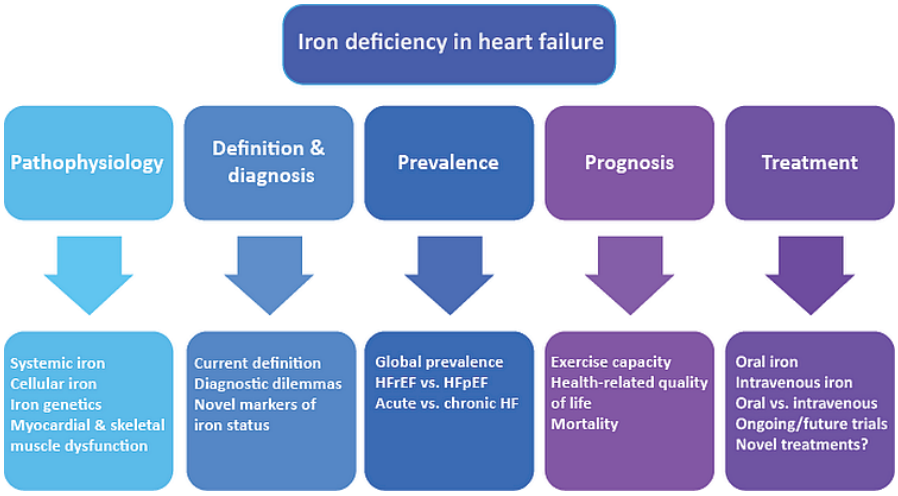

Figure 2. Recent advances made in the field of iron deficiency in heart failure with regard to pathophysiology, classification and definition, prevalence, prognosis and treatment.
Abbreviations: HF = Heart failure, HFpEF = Heart failure with preserved ejection fraction, HFrEF = Heart failure with reduced ejection fraction.

Current insights on iron homeostasis.

Due to its unique ability to shuttle between a ferrous ($Fe2^{+}$) or ferric ($Fe3^{+}$) state, iron is vital for a multitude of fundamental metabolic processes and serves as a key element of proteins with distinct cellular functions.10–13 Traditionally, iron is linked to anemia and the process of erythropoiesis.

Beyond the process of erythropoiesis, iron is indispensable in the uptake and transport of oxygen (component of hemoglobin), oxygen storage (component of myoglobin), maintaining cellular energy and metabolism of extra-hematopoietic tissues (component of oxidative enzymes and respira-

tory chain proteins).[10,13,14] Therefore, maintaining a normal iron metabolism is crucial, especially for cells with high mitogenic potential (e.g. neoplastic, hematopoietic) and high-energy demand (e.g. hepatocytes, skeletal and cardiac myocytes).[7,10,11] This is important for HF, since impaired oxidative metabolism and energy production in both myocardium and peripheral tissues (e.g. skeletal muscle) are cardinal features of this syndrome.10,15

At the other extreme, excess iron accumulates in cells and has the potential to cause deleterious effects. This is mainly through the production of reactive oxygen species (ROS) by the Fenton reaction and results in progressive damage of cellular macromolecules, cell death and tissue injury.16–18 The liver, pancreas and heart are particularly sensitive to iron overload. Therefore, iron homeostasis in the human body has to be firmly regulated to avoid both iron overload and iron deficiency.

The iron cycle

Since humans are unable to excrete iron actively, iron concentration in the body has to be regulated at the site of iron absorption, which mainly takes place in the duodenum (*Figure 2*). The average daily dietary iron intake is 10–20 mg, however only 10% of iron is absorbed and transported across the brush border of duodenal enterocytes.8 Similar amounts of iron are lost from both menstrual and non-menstrual bleeding, and desquamation of epithelial cells in the intestine and the skin.19,20 Whereas heme iron and is efficiently transported by the heme iron transporter, non-heme iron has to be reduced first by brush border ferrireductase, Dcytb, to ferrous iron, the substrate for divalent metal transporter 1 (DMT1).8 In the enterocytic cytosol, iron is either stored as ferritin or exported across the basolateral membrane to the circulation by ferroportin, the only putative transmembrane iron exporter. Efflux of enterocytic iron through ferroportin also requires hephaestin, which oxidizes ferrous iron to ferric iron before it can circulate bound to plasma proteins.21,22 Circulating iron is mainly bound to transferrin which maintains iron in a nonreactive state.20,23 Under normal circumstances, the relative amount of iron bound to transferrin, or transferrin saturation (TSAT), reflects the amount of iron available for metabolizing hematopoietic and non-hematopoietic target cells.

Iron-loaded transferrin is able to deliver its iron into target tissues through a number of mechanisms, but the major pathway of cellular iron import is via transferrin receptor 1 (TfR1)-mediated endocytosis.20,23 Two different transferrin receptors (TfR1 and TfR2) are known, with different expression and regulation. Whereas TfR1 is expressed ubiquitously, TfR2 is mainly

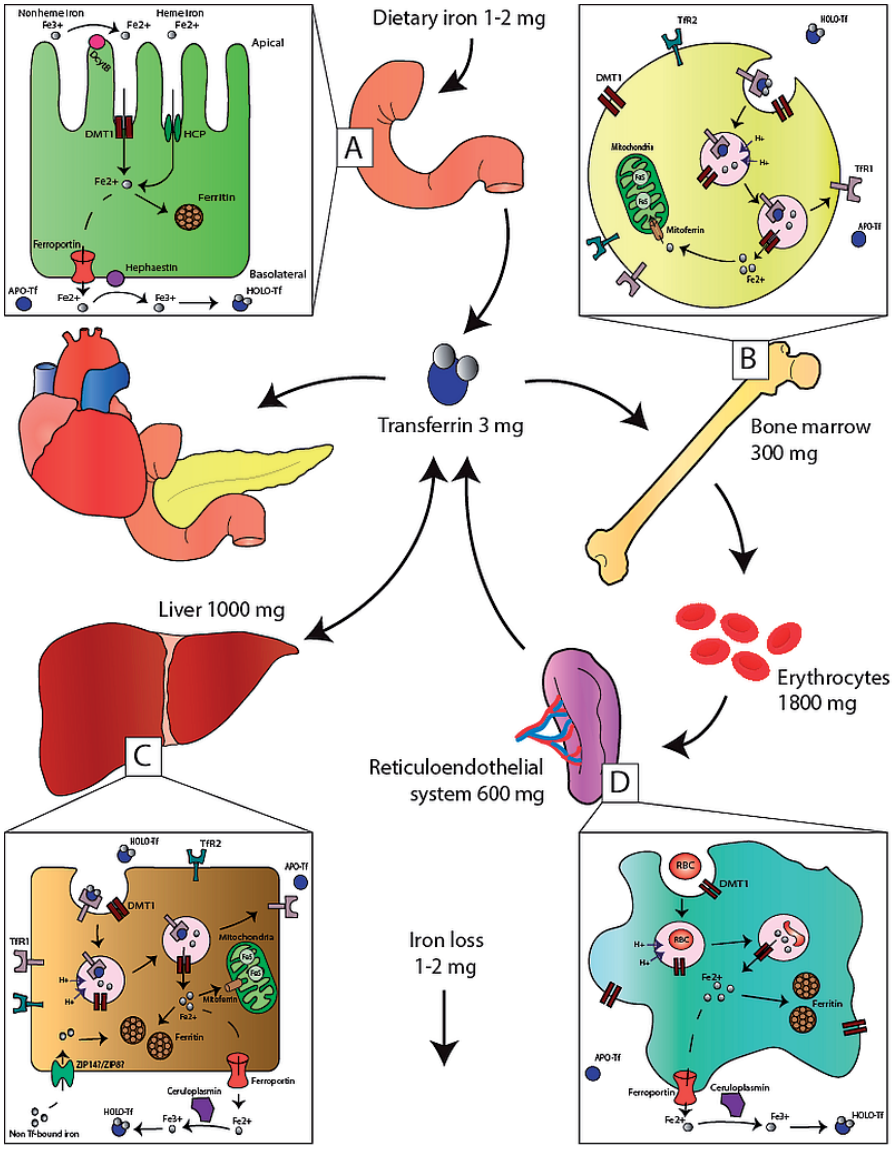

Figure 3. The iron cycle. A: Duodenal enterocyte; B: Erythroid precursor cell; C: Hepatocyte; D: Macrophage of the reticuloendothelial system (RES).

expressed in liver and erythroblasts and has approximately 25-fold lower affinity for iron-bound transferrin. This difference in affinity may therefore enhance the role of TfR2 as an iron sensor, allowing it to be sensitive to changes in transferrin concentration in the blood.24

While only 1–2 mg is compensated by dietary iron absorption, the majority of iron originates from macrophages of the reticuloendothelial system (RES), were the metal is recycled from senescent erythrocytes. Both the RES (mostly the spleen) and the liver are also the major reserve tissues for iron storage in macrophages and hepatocytes in a specialized cytoplasmic iron storage24protein, ferritin.[7,25,26] Relevant to systemic iron homeostasis, ferritin can store large amounts of iron in its interior, with healthy women storing 300–500 mg and men storing 800–1000 mg iron in this way. Under normal circumstances, serum ferritin is a reliable surrogate of storage iron as it closely correlates with body iron stores.[19,20,23,27] In the cytoplasm, iron can be stored as ferritin, used to assemble iron-sulphur clusters (Fe-S), or transported into mitochondria via mitoferrin, were it serves as an essential element in the enzymes of the respiratory chain.28 Mitochondria are the key sites of cellular iron processing where synthesis of both heme and Fe-S clusters take place, but also the generation of ROS.21

Regulators and genetic determinants of iron homeostasis.

Cells and organisms possess carefully regulated but poorly understood mechanisms for iron absorption and metabolism. Hepcidin, a liver-derived peptide and encoded by the gene *HAMP*, has emerged as a key regulator of systemic iron homeostasis. Circulating hepcidin exerts it activity by binding to ferroportin, which triggers its internalization, ubiquitination and subsequent degradation.29 Thus, hepcidin inhibits intestinal iron absorption and impairs the release of recycled iron from macrophages or stored iron in hepatocytes, thereby reducing the availability of iron to target tissues.21,29-31 Whereas depleted iron stores, hypoxia or ineffective erythropoiesis decrease hepcidin expression (and upregulation of iron transport proteins), iron overload and inflammation cause the opposite effect.

Although many studies have examined changes in systemic iron homeostasis in animals and humans, intracellular iron trafficking pathways have only been characterized to some extent. In general, iron regulatory proteins (IRPs) are considered to register cytosolic iron concentrations and post-transcriptionally regulate expression of iron metabolism genes to optimize cellular iron availability.32 In iron-deficient cells, IRPs are thought to bind to iron-responsive elements within the mRNA of ferritin, transferrin, ferroportin and other iron metabolism transcripts, thereby enhancing iron uptake and decreasing iron sequestration.32 However, intracellular iron homeostasis is still not completely understood and warrants further investigation.

Alteration in body iron status is associated with or causes diseases, including anemia and iron overload. Thus, it is important to understand

the causes of variation in iron status, especially information on genetic causes of variation. Recently, a meta-analysis of human genome-wide association study data on biochemical markers of iron status, including up to 48.972 subjects, showed eleven loci with significant effects on one or more of the iron-related phenotypes.33 Six loci showed significant associations with known phenotypes (TF, TFR2, HFE, TMPRSS6) or have known roles in iron homeostasis, including the transferrin receptor TFRC and the iron transporter ferroportin (SLC40A1), while five loci were novel, affected either transferrin (NAT2, ARNTL, FADS2) or ferritin (ABO, TEX14). However, it should be noted that a substantial overlap was observed between loci of iron status and loci affecting erythrocyte and lipid phenotypes.

CLASSIFICATION AND DEFINITION OF IRON DEFICIENCY IN HEART FAILURE

From a practical point of view, iron deficiency can be classified as either absolute or functional. Iron deficiency is absolute when iron stores are reduced or depleted, often with intact iron-regulatory and erythropoietic mechanisms.34,35 Poor nutrition is frequently seen in patients with HF36,37 and increases with disease severity.36 Furthermore, the presence of mucosal edema and a disturbed intestinal microcirculation often results in gastrointestinal malabsorption of iron.38 Iron loss secondary to gastrointestinal side effects caused by concomitant pharmacotherapy (e.g. proton pump inhibitors) may also account for poor iron absorption.38–40 Finally, HF patients using anticoagulation agents or antiplatelet drugs are more prone to bleeding, with consequent chronic microscopic blood loss.41 Persistence of absolute iron deficiency will eventually result in iron deficiency anemia.

In contrast, functional iron deficiency is a state when iron supply is inadequate to meet the body's demands for erythropoiesis, despite normal or elevated body iron stores.42,43 Chronic illness, like HF, often present with augmented generalized inflammation and consecutive activation and production of inflammatory cytokines (e.g. interleukin-6).43,44 This process results in both: (i) a blunted erythropoietin production45,46 and (ii) iron trapping and impaired iron mobilization from cells of the RES to the circulation (mainly driven by hepcidin overproduction). If persistent, the supply of iron to the bone marrow becomes inadequate and anemia of chronic disease typically develops.43,45 This major form of functional iron deficiency is also called iron sequestration. A second type of functional iron deficiency may occur when erythroid marrow is intensely stimulated by endogenous eryth-

ropoietin in response to anemia or in patients treated with erythropoietin stimulating agents (ESA). The increased rate of erythrocyte production outstrips the ability of circulating iron to provide adequate substrate for hemoglobin synthesis. This has been the subject of numerous studies following the widespread use of these agents for treatment of anemia, especially in patients with CKD but recently also in HF.[47,48]

How should iron deficiency be diagnosed in heart failure?

Microscopic examination of bone marrow iron content is still considered the *gold standard* for determining reduced or depleted iron stores.[35] A new bone marrow assessment has been proposed with separate detection of iron in macrophages (stored iron) and erythroblasts (utilized iron), thereby differentiating between iron deficiency states or an overlap of both forms.[49] However, the invasiveness of bone marrow biopsies limit its use in clinical practice and might be replaced by a biomarker-oriented approach to give an indirect overview of the iron status in numerous clinical scenarios.

Since absolute iron deficiency reflects depleted iron stores, its diagnosis is predominantly based on measurements of serum ferritin. In healthy individuals, a cut-off value of < 30 ug/L is proposed to define absolute iron deficiency. As described previously, low-grade inflammation and oxidative stress may artificially increase its concentration in HF, regardless of actual iron status.[34] Therefore, interpreting serum ferritin levels in patients with HF or other chronic illnesses is more challenging. A higher serum ferritin cut-off value (< 100 ug/L) if often used to diagnose absolute iron deficiency in these cases.[34,50]

As frequently seen in patients with chronic diseases, functional iron deficiency is characterized by normal or elevated body iron stores (ferritin levels between 100–299 ug/L). As such, the diagnosis of iron deficiency cannot be made solely on serum ferritin measurements.[34,35] Since this is a problem of supply and demand, and not total body iron deficiency, markers reflecting restricted iron availability may be more useful in this setting.[51] As mentioned above, the relative amount of iron bound to transferrin, or TSAT, reflects the available iron for metabolizing target cells (e.g. erythropoiesis)[34,35] and values below 20% are considered to be associated with suboptimal delivery of iron to target cells.[34,51] However, TSAT has a diurnal variation and, like ferritin, has some acute-phase reactivity. Furthermore, in the setting of chronic disease and malnutrition, transferrin synthesis in the liver is decreased which may artificially elevate the TSAT disproportionate to the actual circulating iron content.[34,52]

Therefore, iron deficiency in HF is classified as absolute when serum ferritin is < 100 ug/L and as functional when serum ferritin 100–299 ug/L with a TSAT < 20%. This definition has been used in clinical HF trials and has been implemented in the most recent HF guidelines of the ESC.[53-55] In addition, a diagnostic work-up for iron deficiency in all patients suspected to have HF is recommended (Class 1, level of evidence C).[55] The 2011 updated Cardiac Society of Australia and New Zealand guidelines for the management of HF contains a grade B recommendation for the evaluation and treatment of iron deficiency in patients with chronic HF. The recent joint HF guidelines of the ACC/AHA have, however, not yet recommended the routine assessment of iron status.[56]

Novel diagnostic criteria for iron deficiency in heart failure?

In recent years, new markers of iron-restricted erythropoiesis have been identified that might aid in the diagnosis of iron deficiency in patients with HF and other chronic illnesses (*Table 1*).[34,57]

Clinically, it would be helpful to detect the earliest changes in red cell indices that reflect iron restricted erythropoiesis instead of examining red cell indices that may be anywhere between 1–120 days. Reticulocytes are the earliest form of erythrocytes released into the circulation and only present for 1–2 days. Accordingly, the amount of hemoglobin in the reticulocytes, or reticulocyte hemoglobin content (CHr), provides a snapshot of how much iron was available for erythropoiesis in a clinically relevant timeframe.[58,59] A reduced CHr < 29 pg is an early signal of IRE and could also serve as an early indicator of response to intravenous iron therapy.[60,61] Whereas the CHr is a real-time parameter (48 hours), the percentage of hypochromic red blood cells (%HYPO) is regarded a time-averaged marker of iron available for erythropoiesis (20–120 days).[62] An increased %HYPO (> 2.5%) is a late indicator of insufficient iron available for erythropoiesis and may be more useful in the diagnosis of iron deficiency.[34,63]

The latest red cells indices to change include hemoglobin, mean corpuscular volume (MCV), mean cell hemoglobin and red cell distribution width (RDW). Mean corpuscular volume is often used to differentiate between different etiologies of anemia. However, its differential power may be limited in patients with HF.[64] Reflecting a quantitative index of anocytosis, the RDW is considered another parameter of iron deficiency. In patients with HF, an elevated RDW is associated with decreased hemoglobin, low MCV, low TSAT and increased morbidity and mortality rates.[65-67] In addition, the RDW is thought to decrease after intravenous iron therapy in HF.[68] However,

Table 1. Biomakers of iron status in heart failure.

Biomarker	Comments
Mean corpuscular volume (MCV) (fL)	Usefull for detecting trends in iron status over periods of weeks or months
	Differential power in HF might be limited
	No use in assessing changes in iron availability secondary to treatment
Red cell distribution width (RDW) (%)	Quantitative index of anocytosis
	Has to be combined with MCV and hemoglobin measurements
	Not only typical for anemia due to iron deficiency, but also other deficiencies (e.g. vitamin B12, folic acid) or sideroblastic anemia
Serum ferritin (ug/L)	Surrogate for iron stores
	Inexpensive measurement
	Acute phase reactant (artificially increased by concomitant inflammatory conditions, infection, neoplasia or liver disease).
Transferrin saturation (TSAT) (%)	Reflects iron availability for metabolizing target cells
	Inexpensive measurement
	Limited by diurnal fluctuations
	A number of clinical disorders can affect transferrin concentrations (artificially increasing TSAT)
Reticulocyte hemoglobin content (CHr) (pg)	Sensitive and early indicator (1–2 days) of iron deficiency.
	Useful in predicting response to intravenous iron therapy
	Can only be measured on a certain types of analyzer
	Local analysis of fresh venous blood is required
Percentage hypochromic red blood cells (%HYPO) (%)	Highly sensitive time-averaged marker of iron-deficient erythropoiesis (20–120 days)
	Might be useful in the diagnosis of iron deficiency
	Can only be measured on a certain types of analyzer
	Red blood cells expand when blood is stored so local analysis of fresh venous blood is required
Soluble transferrin receptor (sTfR) (mg/L)	Good indicator of enhanced erythropoiesis and iron deficiency
	Not affected by inflammatory conditions, but might be by age and ethnicity
	Use is limited by high costs of commercially available assays and lack of international standard
sTfR/ferritin ratio	Proportional to the amount of stored iron in iron-replete patients and tissue iron deficit in iron deficiency
	Limited use in inflammatory conditions due to artificial elevation of ferritin levels independent of iron stores
	Ratio is assay specific
Hepcidin (ng/mL)	Assays in serum and plasma have improved considerably
	Elevated in acute and chronic inflammatory states
	Overestimation of true hepcidin-25 levels due to cross-reaction enzyme-linked immunosorbent assays antibodies
	Mass spectrometry techniques are expensive, labor intensive and time-consuming
	No reference values across different assay platforms

an increased RDW cannot differentiate between types of iron deficiency or possible etiologies of anemia.

A promising parameter of iron status that might be used in the definition of iron deficiency in chronic diseases is the soluble form of the transferrin receptor (sTfR). As a response to an increased iron need by metabolizing target cells, TfR is overexpressed on all cellular membranes (the majority localized on erythroid precursors) and shed to the circulation, which increases sTfR levels.69 Hence, elevated sTfR levels both reflect a higher erythroid proliferation rate and increased iron demand from tissues, but not body iron stores.69 In contrast to ferritin and TSAT, sTfR has been shown to be unaffected by concomitant chronic disease and inflammation.69 Additionally, in a recent study in patients with stable coronary artery disease undergoing cardiac surgery, levels of sTfR serum had the strongest association with bone marrow iron content.70 However, sTfR is a relatively expensive assay and is not widely available.34,51

Hepcidin has emerged as the master regulator of iron status and assays to measure its levels in serum or plasma have improved considerably.71 This has generated hope that determining hepcidin concentrations might be a good alternative to traditional markers of iron status. The main disadvantage of immunoassays is that they detect more than biologically active hepcidin-25 (due to cross-reactivity of the hepcidin antibodies), thereby overestimating true hepcidin levels. On the other hand, mass spectrometric assays are specific for hepcidin-25, but are labor intensive and more expensive.72 Therefore, clinical utility of hepcidin measurements as a diagnostic tool is currently uncertain. Nevertheless, more studies are needed to identify emerging serum markers reflecting iron status (and might differentiate between forms of iron deficiency) with comparison to the criterion standard of bone marrow iron staining in patients with HF.

IRON DEFICIENCY IN HEART FAILURE; GLOBALLY COMMON BUT FREQUENTLY OVERLOOKED

The first reports on the role of iron deficiency in cardiovascular disease date back over 50 years ago.73 Iron deficiency occurred with sympathetic activation, left ventricular hypertrophy, dilatation and symptomatic HF.73–76 Until the early 2000s, iron deficiency in HF was reported to occur up to 20%.77–79 However, these studies mainly focused on lack of iron in the presence of anemia, thereby only reporting the prevalence of absolute iron deficiency. In 148 outpatients with systolic HF and concomitant anemia, Opasich and

colleagues observed that iron deficiency was present in 36% of all anemic patients and in 64% of patients with anemia of chronic disease (defective iron supply for erythropoiesis).45 Only 1 study performed the *gold standard* of bone marrow aspiration in 37 patients with advanced HF and confirmed iron-deprived bone marrow in 27 patients (73%).80 Although serum ferritin was lower in the iron-deficient group compared to patients without iron deficiency, mean ferritin concentration was 113 ug/L, further highlighting the fact that ferritin measurements in patients with HF may not prove reliable in the diagnosis of iron deficiency.80

This makes the definition of iron deficiency in HF not clear-cut and prevalence varies widely depending on the criteria used to detect this condition. When using a more contemporary definition of iron deficiency (ferritin < 100 ug/L or 100–300 ug/L in combination with TSAT < 20%), overall prevalence of 30–50% has been reported in Western populations.[5,81–83] Furthermore, recent studies show similar percentages in patients of African or Asian ethnicity.84,85 Interestingly, in patients with HF and a preserved ejection fraction, the prevalence of iron deficiency might be even higher than 50%.85,86

Studies on the prevalence of iron deficiency in HF have been performed primarily in chronic HF populations. Two recently published studies have reported on the presence of iron deficiency in the acute decompensated phase.87,88 In the CARDIOFER study, Cohen-Solal *et al* examined the prevalence of iron deficiency in 832 chronic HF patients admitted for decompensation.87 Employing the same definition of iron deficiency in the most recent HF guidelines, overall presence was 72% (69% in males and 75% in females) and consisted mainly of absolute iron deficiency. Furthermore, the prevalence of iron deficiency remained high during the entire length of hospitalization.87 Another recent study by Jankowska *et al* investigated the presence of iron deficiency, defined as depleted iron stores (low serum hepcidin) accompanied by increased cellular iron requirements (high serum sTfR), in 165 acute HF patients (31% de novo HF).88 When meeting both conditions (low serum hepcidin and high serum sTfR) iron deficiency was present in 37% of all patients. Moreover, based on based on ferritin and TSAT measurements, iron deficiency was present in 65%, whereas the criteria for both definitions of iron deficiency were fulfilled in 31% of all subjects.88

Despite its high prevalence in both acute and chronic HF, iron deficiency is still not routinely evaluated in these patients and often only explored as a cause of anemia. Silverberg *et al* assessed the degree of clinical awareness of iron deficiency by examining records of 76 patients admitted to the hospital with HF as primary diagnosis.9 In 61 of the 76 patients (80%), there was no or incomplete (e.g. only ferritin or transferrin measurements) iron

workup at the time of admission. In addition, 32 of the 42 anemic patients (76%) had no or incomplete iron work-up,9 suggesting that the link between iron deficiency and HF is not yet known by the majority of physicians treating HF.

IRON DEFICIENCY AND EFFECTS ON CLINICAL OUTCOME IN HEART FAILURE

Conventionally, attention mainly focused on the clinical consequences of inadequate iron supply for erythropoiesis in the context of anemia in HF. However, awareness of the impact of iron deficiency on both morbidity and mortality in HF, irrespective of hemoglobin levels, has been growing over the years. The prognostic role of iron, both within and beyond the process of erythropoiesis, is and has been the subject of interest and focus of several HF studies (*Table 2*).

Dysfunction of myocardium and skeletal muscle

Iron serves as an important co-factor for many proteins, the two major elements that require iron being heme and Fe-S clusters.23 Both heme- and Fe-S cluster dependent enzymes are essential for mitochondrial energy metabolism. Moreover, a clear U-shaped relationship between the amount of mitochondrial iron and energy metabolism has been described; both insufficient and excessive iron can cause significant mitochondrial dysfunction.89 When cellular iron is depleted, a reduction in Fe-S cluster activity is triggered,90 causing a reduced mitochondrial ATP production during the citrate cycle and consecutive exercise intolerance and fatigue. On the other hand, iron overload can induce or exacerbate cardiomyopathy, mainly through ROS-dependent mechanisms and disruption of Fe-S cluster biogenesis.91,92

Previously, it has been demonstrated that iron deficiency in animal models leads to the development of cardiac fibrosis,93 increased sympathetic activation, and progressive left ventricular dysfunction.74–76 The observations made in animal studies highlight that iron uptake and intracellular iron trafficking in the myocardium may be critical for cardiac function. In the failing human myocardium, Maeder and colleagues also observed more iron depletion compared to non-failing hearts.94 This was demonstrated by a reduced myocardial iron content as well as a significant reduction in mRNA expression of TfR1.94 Two recently published studies, using cardiac magnetic resonance imaging to quantify myocardial iron content in patients with HF, showed that lower myocardial iron was associated with an increased risk

Table 2. Studies on iron deficiency and clinical outcome in heart failure.

Study outcome	Study design	N	Study population	Definition iron deficiency	Iron deficient (%)	Main findings
Quality of life	Post-hoc analysis of single- center data97	552	Chronic HF with reduced (≤ 45%) or preserved LVEF	Ferritin < 100 ug/L or < 800 ug/L with TSAT < 20%	63%	Iron deficiency was associated with worse health-related QoL (using MLHFQ) and physical dimension score
	Pooled cohort analysis (3 countries)98	1278	Chronic HF with reduced (≤ 45%) or preserved LVEF	Ferritin < 100 ug/L or < 100–299 ug/L with TSAT< 20%	58%	Iron deficiency, and not anemia, was associated with worse health-related QoL (using MLHFQ)
Exercise capacity	Prospective two-center95	443	Chronic HF with reduced (< 45%) LVEF	Ferritin < 100 ug/L or < 100–299 ug/L with TSAT< 20%	35%	ID, and not anemia, was associated with reduced peak VO2 and increased VE/VCO2 slope in multivariable analysis
	Prospective, two-center96	27	Chronic HF with reduced (≤ 45%) LVEF	TSAT < 20%	43%	Iron deficiency correlated with lower peak VO2 and higher VE/CO2 slope (adjusted for NYHA class and hemoglobin)
	Prospective single-center91	26	Chronic HF with preserved LVEF (no cut-off value in article)	Ferritin < 100 ug/L or < 100–299 ug/L with TSAT < 20%	58%	No association between iron deficiency and exercise capacity (peak VO2 or VE/VCO2 slope)
	Post-hoc analysis of multi- center data90	48	Chronic HF	Ferritin < 100 ug/L or < 100–299 ug/L with TSAT < 20%	61%	TSAT, but not ferritin levels, correlated with peak VO2 in multivariable analysis
Mortality	Prospective, two-center 86	546	Chronic HF with reduced (≤ 45%) LVEF	Ferritin < 100 ug/L or < 100–300 ug/L with TSAT < 20%	37%	Iron deficiency, and not anemia, was associated with an increased risk for mortality or heart transplantation
	Prospective two-center [96]	157	Chronic HF with reduced (≤ 45%) LVEF	TSAT < 20%	43%	Iron deficiency with or without anemia was associated with a 2- and 4-fold greater risk for death, respectively

Table 2. (continued)

Study outcome	Study design	N	Study population	Definition iron deficiency	Iron deficient (%)	Main findings
Mortality	Community-based survey [87]	547	Self-reported community dwelling HF patients	Ferritin < 100 ug/L or < 100–300 ug/L with TSAT < 20%	61%	No association between iron deficiency and cardiovascular or all-cause mortality
	Pooled cohort analysis (3 countries) [5]	1506	Chronic HF with reduced (≤ 45%) or preserved LVEF	Ferritin < 100 ug/L or < 100–300 ug/L with TSAT < 20%	50%	Iron deficiency was associated with an increased risk for death
	Retrospective single-center [100]	274	Chronic HF with reduced (≤ 45%) LVEF	Progression of iron deficiency was defined as increasing RDW with decreasing mean cell volume	23%	Evolving iron deficiency was related to higher rates of mortality
	Prospective, single-center [88]	127	Chronic HF with reduced (≤ 45%) LVEF	Ferritin < 100 ug/L or < 100–299 ug/L with TSAT < 20%	36%	Iron deficiency, and not anemia, was associated with an increased risk for the composite endpoint of all-cause mortality and non-fatal cardiovascular events
	Post-hoc analysis of multi- center data [90]	751	Chronic HF	Ferritin < 100 ug/L or < 100–299 ug/L with TSAT < 20% TSAT < 20% (functional ID)	61%	TSAT < 20% with or without anemia, was associated with an increased risk for all-cause mortality and HF readmissions
	Prospective two-center [93]	165	Acute HF	Low hepcidin (< 14.5 ng/mL) and high sTfR (≥ 1.59 mg/L) Ferritin < 100 ug/L or < 100–299 ug/L with TSAT < 20%	37% (based on hepcidin/sTfR) 65% (based on ferritin/TSAT)	Depleted iron stores (low hepcidin) and unmet cellular iron requirements (high sTfR) were associated an increased risk for all-cause mortality.

Abbreviations: HF = Heart failure, LVEF = Left ventricular ejection fraction, MLHFQ = Minnesota living with heart failure questionnaire, QoL = Quality of life, sTfR = Soluble transferrin receptor, TSAT = Transferrin saturation.

for major adverse cardiac events.95,96 Additionally, myocardial iron depletion was more often observed in non-ischemic HF compared to ischemic HF or patients with normal left ventricular function.95 Furthermore, a third study evaluating myocardial iron deposition in HF patients using T2* magnetic resonance imaging on multiple time points is currently recruiting (NCT02527330; n=100).

Persisting iron deficiency is also associated with exercise intolerance (both a decrease in endurance capacity and maximal performance). In subjects without HF, a causal relationship between iron deficiency and impaired exercise capacity has been clearly described, both with and without reduced hemoglobin levels.97 In patients with HF, iron deficiency with and without anemia, has also been associated with impaired exercise capacity.98 In a prospective trial comprising 443 patients with stable systolic HF, iron deficiency - and not anemia - was associated with exercise intolerance (expressed as a decreased peak oxygen production [peak VO2] and higher ventilatory response to exercise [VE/VCO2 slope]). This relationship remained after adjustment for clinical covariates and was observed in both anemic and non-anemic patients.98 These data confirmed findings from a previous prospective study in 27 HF patients, were iron-depleted patients had a lower peak oxygen consumption and higher ventilatory response to exercise compared to iron-replete patients.99 When ejection fraction is preserved, no significant relationship between iron deficiency and exercise capacity has been observed.86

Iron deficiency and quality of life in heart failure

Patients with HF often report a substantial impairment of their health-related quality of life (HRQoL) compared to normal populations and to patients with other chronic conditions.2 In a post-hoc analysis of a cohort of 552 HF patients, Comin-Colet and colleagues evaluated the influence of iron deficiency on HRQoL.100 Again, iron deficiency - and not anemia - remained an independent predictor of worse HRQoL, mostly due to physical limitations.100 Additionally, higher sTfR levels were also independently related to worse HRQoL in this study. Results from a international multicenter study, comprising 1278 patients with HF, confirmed the aforementioned association between iron deficiency and worse HRQoL, independent of the presence of anemia or whether ejection fraction was reduced of preserved.101

Iron deficiency and outcome in heart failure

Several studies have focused on the association between iron deficiency and mortality in chronic HF with reduced ejection fraction.[5,81–83,99,102,103] In

most studies, the presence of iron deficiency was associated with increased mortality after adjustment for confounding factors.[5,81,83,99,102,103] Regardless of the severity of HF, this association was observed in iron deficiency with or without concomitant anemia and/or CKD.102 Only one recently published study has investigated the prognostic role of iron in acute HF.88 Multivariable analyses revealed iron deficiency, defined as low-serum hepcidin and high-serum sTfR, to be associated an increased risk for 12-month all-cause mortality compared to patients with a preserved iron status.88 Whether iron deficiency has similar detrimental effects in HF with preserved ejection fraction is currently unknown.

MANAGEMENT OF IRON DEFICIENCY IN HEART FAILURE

Although the correction of anemia was thought of as an attractive and novel treatment approach in HF, as shown in a meta-analysis of small studies,104 the neutral results of the large pivotal RED-HF trial48 suggest that anemia might not be a mediator of outcome, but more likely a marker of disease severity. Given the aforementioned high prevalence and unfavorable effects of iron deficiency, it seems plausible to attempt to correct this disorder by repletion of iron. Indeed, current available evidence suggests that treatment of iron deficiency appears to confer benefit in patients with HF, whereas strategies solely aimed to improve hemoglobin do not.

Oral iron supplementation

Repletion with oral iron supplements is initially given to patients with iron deficiency. Different types of oral iron salts, such as iron sulphate or iron gluconate, have been used to treat iron deficiency. Oral iron is relatively inexpensive and therefore widely used. Despite its practical advantages, intolerance to oral iron, due to gastrointestinal side effects, as well as a number of drug interactions (e.g. proton pump inhibitors) are frequently observed and may lead to limited compliance. Moreover, iron absorption is low when administered orally, especially in patients with HF or CKD. Achieving iron repletion (estimated in a range exceeding 1000 mg)105,106 using oral iron may therefore take much longer in patients with HF, also with the risk of poor tolerance and compliance. Nonetheless, minimal evidence for the use of oral iron supplementation in the setting of HF exists and no randomized trial has investigated oral iron versus no iron therapy without adjuvant ESA therapy. The oral Iron Repletion effects ON Oxygen UpTake in Heart Failure (IRON-OUT; NCT02188784; n = 220), a large multicenter, random-

ized, placebo-controlled trial is expected to start soon and will investigate the role of oral iron polysaccharide on change in peak VO2 in systolic HF patients with iron deficiency.

Intravenous iron supplementation

Intravenous iron therapy has been recently advocated in HF and might be considered for iron administration in patients with iron deficiency.55 The fact that it bypasses the absorption difficulties associated with oral iron supplementation, makes it an interesting treatment approach in HF or CKD. On the other hand, serious side-effects, particularly hypersensitivity reactions, have been described, however, mainly reported for iron dextran. Newer preparations, such as ferric carboxymaltose (FCM) or iron isomaltoside 1000, do not seem to produce these reactions and thereby permit a higher dose in a single administration.107–109

In patients with HF, intravenous iron supplementation to maintain adequate iron levels was first used as part of a treatment regimen with ESAs in two studies.110,111 However, in both studies the effect of iron in combination with ESAs was examined and not the effect of intravenous iron alone. The efficacy and safety of intravenous iron supplementation alone as a treatment approach for iron deficiency in HF has been the subjects of examination in six studies, of which two open-label uncontrolled trials and four randomized, placebo-controlled studies (*Table 3*).[53,54,106,112–114] Two single-center open-label trials showed beneficial effects of iron sucrose in terms of hemoglobin levels and iron parameters as well as several cardiac endpoints including NYHA functional class, ejection fraction and exercise capacity.112,114 Two smaller randomized studies, also administering iron sucrose, demonstrated improved exercise capacity, symptom severity, cardiac function and quality of life.53,113 These benefits were observed in both anemic and non-anemic patients at baseline, although greater in anemic patients.53 In a recent update of one of the aforementioned randomized controlled trials, treatment with intravenous iron was associated with improved systolic and diastolic function after 6 months, but not in the placebo group.115

The Ferinject Assessment in Patients with Iron Deficiency and Chronic Heart Failure (FAIR-HF) study is the largest randomized, placebo-controlled trial to date enrolling 459 HF patients with iron deficiency (ferritin < 100 ug/L or 100–299 ug/L with a TSAT < 20%) and with or without anemia (hemoglobin 9.5–13.5 g/dL).54 Patients received either intravenous FCM or placebo in a 2:1 ratio. After 24 weeks of follow-up, beneficial effects of intravenous iron supplementation on both primary endpoints (patient's global assessment and NYHA functional class) were observed compared to

placebo.54 Secondary endpoints, including changes in 6-minute walking test (6MWT) and HR-QoL, also improved significantly in favor of the FCM group. Interestingly, in this study and a subanalysis of FAIR-HF, improvements in both primary and secondary endpoints were observed regardless whether the patients was anemic or nonanemic, suggesting that iron deficiency can be treated independent of low hemoglobin levels.54,105 A recent post-hoc analysis of the FAIR-HF also suggests that treatment with intravenous iron improves renal function.116 Even though FAIR-HF was not designed to test the effect of intravenous iron therapy on outcome and follow-up was limited at 6 months, safety endpoints were similar between groups, but a trend towards fewer cardiovascular hospitalizations was observed in the IV iron group.54

To support these findings and evaluate the benefits and safety of long-term intravenous iron therapy, the recently published CONFIRM-HF (Ferric CarboxymatOse evaluation on perFormance in patients with IRon deficiency in coMbination with Heart Failure) trial enrolled 304 ambulatory symptomatic HF patients with iron deficiency.106 Patients were randomized 1:1 to either FCM (n = 152) or placebo (n = 152) and follow-up was 52 weeks. Primary endpoint of the CONFIRM-HF was the change in 6MWT from baseline to week 24. Similar to the FAIR-HF study, the CONFIRM-HF study showed significant improvements in both primary and secondary endpoints (patient global assessment, NYHA functional class, HR-QoL) in favor of the FCM group.106 Additional secondary outcome-related endpoint analyses showed similar findings between both groups in terms of mortality, however a significant reduction the risk of hospitalizations due to worsening HF was observed in the intravenous iron group compared to placebo [hazard ratio (95% confidence interval): 0.39 (0.19 – 0.82), P = 0.009].106

It should be noted that all trials investigating the effect of intravenous iron as the sole treatment for iron deficiency in HF have enrolled patients with HF and a reduced ejection fraction. The safety and efficacy of intravenous iron supplementation in patients with HF and a preserved ejection fraction has not been investigated. Furthermore, it remains unclear whether intravenous iron treatment also has beneficial effects on morbidity and mortality.

Oral versus intravenous iron therapy in heart failure

A number of studies in patients with CKD have shown that intravenous iron may be superior over oral iron supplementation when also receiving ESAs.[117–120] One study compared intravenous to oral iron in 75 non-dialysis CKD patients who were not receiving concomitant ESA therapy.121 The mean

Table 3. Overview of randomized controlled trials on intravenous iron supplementation in patients with heart failure.

Study	Population	N*	Duration	Treatment regimen	Definition of iron deficiency	Endpoints (versus controls)
Toblli et al. 2007 [110]	Iron deficiency with hemoglobin < 12.5 g/dL, reduced (≤ 35%) LVEF and creatinine clearance < 90 ml/min	40	26 weeks	Iron sucrose 200 mg/week for 5 weeks versus placebo	Ferritin < 100 ug/L and TSAT < 20%	Reduction in NT-proBNP and CRP levels. Improvement of LVEF, renal function, NYHA class, exercise capacity|| and QoL (MLHFQ) (all p< 0.01)
Okonko et al. 2008 FERRIC-HF [61]	Iron deficiency with or without anemia, NYHA class II-III, reduced (≤ 45%) LVEF, exercise limitation (peak VO2 < 18 ml/kg/min)	35	18 weeks	Iron sucrose 200 mg/week until ferritin > 500 ug/L versus no treatment[†]	Ferritin < 100 ug/L or 100–300 ug/L with TSAT < 20%	Trend to increase in VO2 max (p=0.08). Significant Significant improvement in VO2 max/kg (p=0.01). Improvement in NYHA class and PGA (all p< 0.01)
Anker et al. 2009 FAIR-HF [62]	Iron deficiency with or without anemia, NYHA class II-III	459	24 week	Ferric carboxymaltose 200 mg/weekly until iron repletion[‡] versus placebo	Ferritin < 100 ug/L or 100–299 ug/L with TSAT < 20%	Improvement in NYHA class and PGA (all p < 0.01). Improvement in exercise capacity|| and QoL (EQ-5D and KCCQ) (all p< 0.001).Similar effects in anemic and nonanemic patients
Ponikowski et al. 2014 CONFIRM-HF [103]	NYHA class II-III, with or without anemia, reduced (≤ 45%) LVEF, elevated natriuretic peptides and hemoglobin < 15 g/dL	304	52 weeks	Ferric carboxymaltose 500–2000 mg in therapy phase (baseline/week 6) during maintenance phase[§] versus placebo	Ferritin < 100 ug/L or 100–300 ug/L with TSAT < 20%	Improvement in exercise capacity[||] at 24 weeks and 52 weeks. Improvement in NYHA class, PGA, fatigue score and QoL (EQ-5D) from week 24 onwards (all p< 0.01). Reduction in in risk of HF hospitalizations (p< 0.01)#

*2:1 randomization, 24 of 35 in FERRIC-HF study and 304 of 459 in FAIR-HF study on active drug. Other studies had a 1:1 randomization. [†]Until ferritin levels > 500 ug/L, then 200 mg iron/month thereafter. [‡]Until iron repletion was complete, then every 200 mg every 4 weeks to week 24. [§]Only maintenance dosing of 500 mg ferric carboxymaltose if iron deficiency was still present re-assessed each visit. [||]Assessed using six-minute walk test. [#]Not powered for morbidity/mortality analyses.
Abbreviations: KCCQ = Kansas City cardiomyopathy questionnaire, LVEF = Left ventricular ejection fraction, MLHFQ = Minnesota living with heart failure questionnaire, NYHA = New York Heart Association, PGA = Patient global assessment, TSAT = Transferrin saturation.

change in hemoglobin concentration from baseline did not significantly differ between treatment groups, whereas the mean change in TSAT or ferritin concentration from baseline significantly differed between both groups and in favor of the IV iron group.121 The authors concluded that oral and intravenous iron resulted in similar increases in hemoglobin concentrations in iron-deficient CKD patients not receiving ESAs, although in comparison to oral iron, intravenous iron may result in a more rapid repletion of iron stores. It should be noted that in this study a greater number of patients in the intravenous iron group experienced adverse effects (29.5% versus 20% in the oral iron group).121

To date, only one pilot study compared oral versus intravenous iron supplementation in terms of efficacy and safety in patients with HF. The IRON-HF trial comprised 23 patients with HF (NYHA class II-IV), a reduced ejection fraction (< 40%), anemia (9–12 g/dL) and iron deficiency (ferritin < 500 ug/L and TSAT < 20%).122 Patients were randomized to either IV iron sucrose (200 mg once a week for 5 weeks), oral ferrous sulphate (3 times a day 200 mg) for 8 weeks, or placebo. The primary endpoint was the variation in peak oxygen consumption (VO2) over 3-month follow-up, and was available in 18 patients. A clinically relevant difference of 4.36 ml/kg/min in peak VO2 was observed between the IV iron group (+3.5 ml/kg/min) and oral iron group (-0.86 ml/kg/min), although not significantly different.122 However, the original trial terminated quite short of its intended sample size (119 patients). Therefore, these findings are underpowered to detect any difference between the groups and should be interpreted with caution. A definitive prospective head-to-head comparison between oral and IV iron supplementation in HF patients is still pending.

IMPLICATIONS FOR CLINICAL PRACTICE AND FUTURE DIRECTIONS

A number of trials, ongoing or expected to start soon, will further investigate on the role of iron therapy in HF on multiple endpoints of interest (*Table 5*). Moreover, studies targeting the expression of regulators of systemic (e.g. hepcidin antagonists) or cellular (e.g. mitochondrial iron therapy) iron homeostasis might be of interest as possible future treatments approaches.

Ongoing and future trials

The Effect of Ferric Carboxymaltose on Exercise Capacity in Patients with Iron Deficiency and Chronic Heart Failure (EFFECT-HF; NCT01394562), a

Table 4. Ongoing and future studies on treatment of iron deficiency in heart failure.

Study	Status	Design	N	Duration	Patient population	Definition of iron deficiency	Treatment	Primary endpoint
EFFECT-HF	Recruiting	Randomized, open label	160	24 weeks	NYHA II-III, LVEF ≤ 45%, BNP/NT-proBNP > 100/> 400 pg/ml, peak VO2 10–20 ml/kg/min	Ferritin < 100 ug/L or 100–300 ug/L with TSAT < 20%	IV FCM vs. standard of care	Change in peak VO2 by cardiopulmonary exercise testing
Active Working Group Romania	Recruiting	Randomized, open label	200	12 months	NYHA III, LVEF ≤ 40%, Hb < 12 g/dL, eGFR 30–59 ml/min/1.73m2	Ferritin < 100 ug/L or 100–300 ug/L with TSAT < 20%	IV iron sucrose vs. standard of care	Change in LVEF
PRACTICE-ASIA-HF	Recruiting	Randomized, single-blind, placebo-controlled	50	12 weeks	Hospitalized for HF (regardless of LVEF), Hb < 14 g/dL,	Ferritin < 300 ug/L with TSAT < 20%	IV FCM vs. placebo	Change in 6MWT
iCHF	Recruiting	Randomized, double-blind, placebo-controlled	100	12 weeks	NYHA II-III, LVEF ≤ 45%, Hb 9.5–13.5 g/dL	Ferritin < 100 ug/L or 100–300 ug/L with TSAT < 20%	IV FCM vs. placebo	Change if LVEF (cardiac MRI)
Pilot study University of Zurich	Recruiting	Randomized, double-blind, placebo-controlled	20	12 weeks	LVEF ≤ 40% (NYHA II) or LVEF ≤ 45% (NYHA III), Hb 9.5–13.5	Ferritin < 100 ug/L or 100–300 ug/L with TSAT < 20%	IV FCM vs. placebo	Effect of FCM on mitochondrial gene activation pattern
IRON-OUT	Recruiting	Randomized, double-blind, placebo-controlled	220	16 weeks	LVEF ≤ 40%, Hb 9.5–13.5	Ferritin < 100 ug/L or 100–300 ug/L with TSAT < 20%	Oral polysaccharide iron complex vs. placebo	Change in peak VO2 by cardiopulmonary exercise testing
FAIR-HFpEF	Not yet recruiting	Randomized, double-blind, placebo-controlled (1:1)	260	52 weeks	NYHA II-III, LVEF > 45% and diastolic dysfunction, BNP/NT-proBNP > 100/> 400 pg/ml, MR-proANP > 125 pmol/L, Hb > 9.0-≤ 14 g/dL	Ferritin < 100 ug/L or 100–300 ug/L with TSAT < 20%	IV FCM vs. placebo	Change in 6MWT

Abbreviations: 6MWT = Six-minute walking test, FCM = Ferric carboxymaltose, Hb = Hemoglobin, HF = Heart failure, IV = Intravenous, LVEF = Left ventricular ejection fraction, MRI = Magnetic resonance imaging, MR-proANP = Mid-regional pro-atrial natriuretic peptide, NYHA = New York Heart Association, (NT-pro)BNP = (N-terminal pro) Brain-type natriuretic peptide, TSAT = Transferrin saturation.

randomized, open-label trail is ongoing and investigating the effect of treatment with intravenous iron versus standard of care on exercise capacity (change in peak VO2 from baseline to 24 weeks), symptoms and quality of life in patients with systolic HF (LVEF ≤ 45%) and iron deficiency. The Iron in Congestive Heart Failure (iCHF; NCT01837082) trial is an randomized, double-blind, placebo-controlled trial focuses on the effect of IV iron on change in both LVEF (measured by cardiac magnetic resonance imaging) and glomerular filtration rate (detected by radionuclide Chromium-51-EDTA) from baseline to 12 weeks in systolic HF (LVEF ≤ 45%). The FAIR-HFpEF trial is the first randomized, double-blind, placebo-controlled study to examine the role of intravenous iron on change in exercise capacity solely in patients with HF and a preserved ejection fraction HF and is scheduled to commence in 2015. A pilot study, currently recruiting, is looking at the effect of IV iron in Asians hospitalized for HF, regardless of LVEF (PRACTICE-ASIA-HF; NCT01922479). Another pilot study is examining the effect of FCM on mitochondrial gene activation pattern after 12 weeks of treatment (NCT01978028). Finally, one study, set to investigate the effects of treatment with IV FCM on morbidity and mortality in HF patients with reduced ejection fraction, is currently underway (FAIR-HF 2).

Mitochondrial iron therapy

The mechanisms of heart failure are still not quite understood, but it appears that mitochondrial dysfunction is a critical factor in this illness. Although systemic iron homeostasis has been extensively studied in both animals and humans, the role of mitochondrial iron in the field of HF has only been characterized to some extent. Despite this, indirect evidence points towards potentially beneficial therapeutic implications of altering mitochondrial iron homeostasis in the failing heart. It should be noted that most studies on mitochondrial iron therapy have been in the setting of iron overload. However, given the previous described U-shaped relationship between iron levels and mitochondrial function, one could speculate that treatment of iron deficiency may also improve mitochondrial function.93 Nonetheless, the role of mitochondrial iron homeostasis in the setting of iron deficiency merits further investigation and more focus on targeting mitochondrial dysfunction is warranted.

Hepcidin antagonists

Hepcidin might be a prime target for regulation of systemic iron homeostasis in the body. Although the regulation of hepcidin expression is complex, a number of studies, both *in vitro* and *in vivo*, have investigated the role

of potential molecular targets to inhibit hepcidin expression.123 A hepcidin-neutralizing antibody has already been successfully used in a mouse-model. (ref) Furthermore, the recently discovered protein erythroferrone is thought to act as an erythroid regulator by suppressing hepcidin expression, thereby allowing more iron to be made available for erythropoiesis.124 Other agents targeting pathways that regulate hepcidin expression have also been described. Dorsomorphin and soluble hemojuvulin, both inhibitors of the hepcidin signaling pathway, have been shown to decrease hepcidin expression in vivo. Finally, existing therapies, such as anti-IL-6 antibodies, suppress hepcidin production and improve anemia. Undoubtedly, future studies are needed to assess whether hepcidin could be useful to establish an indication for anti-hepcidin therapy, or whether it could be helpful in monitoring anti-hepcidin therapy.

CONCLUSIONS

Iron is an indispensable element for the human body, both within and beyond the process of erythropoiesis. Patients with HF are prone to become iron deficient and constitutes a globally frequent comorbidity in HF, not only as a factor leading to anemia, but also as a separate condition with adverse clinical and prognostic consequences. Conventional markers in the assessment of iron status have certain limitations in HF or other chronic diseases. Therefore, more studies are needed to identify potentially new and/or additional serum markers reflecting iron status with comparison to the gold standard of bone marrow iron staining in patients with HF. Although the pathophysiology of iron deficiency in HF in still partially unclear, a number of clinical trials have demonstrated that treatment with IV iron has favorable effects on NYHA functional class, exercise capacity, renal function, echocardiographic parameters, and quality of life. Left unanswered is whether treatment affects outcomes and if intravenous iron supplementation is equally beneficial in patients with HF and a preserved ejection fraction. Furthermore, a definitive head-to-head comparison between oral and IV iron supplementation in HF is still pending. Ongoing and future studies are warranted to finally establish whether abnormal iron homeostasis may become one of the treatable aberrant metabolic pathways in the HF syndrome.

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